25. (Reiterated) The pegylated IFN-α2 of claim 24, wherein the PEG moiety is attached to a region proximal to Helix A or a C-D loop of IFN-α2.

26. (Reiterated) A multimeric protein comprising at least two proteins each having a free cysteine and wherein said proteins are attached through said free cysteines.

27. (Reiterated) A method of treating a condition treatable with growth hormone, EPO or an alpha interferon, comprising administering to a patient in need thereof an effective amount of a cysteine variant of growth hormone, EPO or alpha interferon, or a derivative thereof to treat said condition.

28. (Reiterated) The method of claim 27, wherein said derivative is a pegylated cysteine variant of growth hormone, EPO or alpha interferon.

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29. (Once Amended) A method for covalently modifying a protein produced according to claim 1, comprising the steps of:

(a) purifying the soluble protein;

(b) reducing the protein with a disulfide-reducing agent; and

(c) exposing the protein to a cysteine-reactive moiety to obtain a cysteine-modified protein.

30. (Reiterated) The method of claim 29, further comprising isolating the cysteine-modified protein from the unmodified protein.

31. (Reiterated) The method of claim 29, wherein the cysteine-reactive moiety is a polyethylene glycol.

REMARKS

The above amendments are being submitted in connection with the national stage filing of the present application. Claim 1 has been amended to clarify that the step of exposing the soluble protein to a cysteine blocking agent occurs prior to the actual isolation of the soluble protein from the cell. Support for this amendment is found on page 10, line 24, to page 11, line 3 of the specification. In addition, Claim 1 has been amended to clarify that the cysteine blocking agent of the present invention is an agent that forms a mixed disulfide with a cysteine in the soluble protein. Support for this amendment is found on page 11, lines 14-17 of the specification. Claim 2 has been

amended to clarify the language with regard to Claim 1. These amendments were originally timely filed in the corresponding international application (PCT/US00/00931), but it is believed that the PCT Office failed to deliver the amendment to the Examiner prior to the issuance of the Preliminary Examination Report. Therefore, the amendments are presented again here by way of Preliminary Amendment. The remaining amendments eliminate the multiple dependent claims from the application.

Respectfully submitted,

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Marked-Up Version Showing Amendments

Claims 1, 2, 4, 5, 7, 18, 19 and 29 have been amended as shown below. Claims 3, 6, 8-17, 20-28 and 30-31 are pending and have not been amended.

1. (Amended) A method for obtaining a soluble protein having a free cysteine comprising the step of:

a. obtaining a host cell capable of expressing the soluble protein;

b. exposing the host cell to a cysteine blocking agent prior to step (c), wherein said cysteine blocking agent forms a mixed-disulfide with at least one cysteine residue in said soluble protein; and

c. isolating the soluble protein from the host cell.

2. (Amended) The method of claim 1, [further comprising] wherein said step (b) of exposing comprises disrupting the host cell in the presence of the cysteine blocking agent, and wherein said step (c) of isolating comprises [and] isolating the protein from the soluble fraction of the disrupted host cell.

4. (Once Amended) The method of [claims] claim 1 [to 3], wherein said host cell is a bacteria, yeast, insect or mammalian cell.

5. (Once Amended) The method of [claims] claim 1 [to 3], wherein said host cell is a bacteria cell.

7. (Once Amended) The method of [claims] claim 1 [to 3], wherein said soluble protein is a recombinant protein.

18. (Once Amended) The method of [claims] claim 1 [to 3], further [comrpsiing] comprising attaching a cysteine-reactive moiety to said isolated protein to form a cysteine modified protein.

19. (Once Amended) The method of [claims] claim 1 [to 3], further comprising attaching a polyethylene glycol to said isolated protein to form a pegylated protein.

29. (Once Amended) A method for covalently modifying a protein produced according to [claims] claim 1 [to 3], comprising the steps of:

(a) purifying the soluble protein;

(b) reducing the protein with a disulfide-reducing agent; and

(c) exposing the protein to a cysteine-reactive moiety to obtain a cysteine-modified protein.